



Irrigation Frequency and Leaf Whorl Position Jointly Regulate Axillary Bud Quality of *Hevea brasiliensis* ‘Reken 628’

Wenping Li^{1,2}, Huizhen Yang^{1,2}, Xianhong Chen^{1*}, Jun Wang¹

¹ Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan, China

² College of Tropical Crops, Yunnan Agricultural University, Pu'er, Yunnan, China

Email: chenxhongjane@163.com (X. Chen).

*Corresponding author

Received: 18 Mar 2026; Received in revised form: 16 Apr 2026; Accepted: 22 Apr 2026; Available online: 30 Apr 2026

©2026 The Author(s). Published by Infogain Publication. This is an open-access article under the CC BY license

(<https://creativecommons.org/licenses/by/4.0/>).

Abstract—To clarify the regulatory effects of irrigation frequency and leaf whorl position on axillary bud quality of rubber tree ‘Reken 628’, a two-factor split-plot experiment was conducted using 3-year-old single-stem bud sticks. Two irrigation frequencies (once daily and twice daily) and three leaf whorl positions (2nd, 3rd, 4th reverse leaf whorls) were set. Morphological and physiological indices were measured, followed by ANOVA, correlation analysis, TOPSIS and PCA. The results showed that irrigation frequency had significant effects on leaf water content and scale bud eye length, with a significant interaction between irrigation frequency and leaf whorl position. Leaf width was extremely significantly positively correlated with leaf water content, and leaf water content was extremely significantly negatively correlated with scale bud scar thickness. TOPSIS evaluation showed that the highest axillary bud quality was observed under twice daily irrigation + 2nd reverse leaf whorl, with a comprehensive index of 0.7785. Under the same leaf whorl, twice daily irrigation was significantly better than once daily irrigation. This study provides a technical basis for standardized cultivation of ‘Reken 628’ bud sticks.



Keywords—*Hevea brasiliensis*, irrigation frequency, leaf whorl, axillary bud quality, TOPSIS

1. INTRODUCTION

Hevea brasiliensis is a major economic tree species in tropical China. Budding propagation is central to the large-scale deployment of improved rubber tree cultivars, and axillary bud quality directly determines budding survival rate and seedling vigor, thus representing a key factor governing the efficiency of improved variety breeding. ‘Reken 628’ is a high-yielding stress-tolerant, and widely adaptable rubber tree cultivar that has been increasingly planted in major production regions including Hainan and Yunnan[1]. This cultivar features a compact canopy and pronounced natural self-pruning, which improves understory light transmission and favors intercropping systems[2]. Establishing optimized cultivation techniques for high-quality bud sticks is therefore practically important.

Water is a key environmental factor regulating plant organ development. Irrigation frequency modulates axillary bud differentiation and morphogenesis by influencing plant water status, photosynthate translocation, and cell turgor. Leaf whorl position determines source-sink strength and nutrient supply to axillary buds, leading to substantial variation in axillary bud quality among positions. Current research on rubber tree axillary bud quality has focused on single factors such as bud type[3], rootstock[4], leaf management[5–6], and light intensity[7]. However, the interactive effects of irrigation frequency and leaf whorl position on axillary bud quality in ‘Reken 628’ remain poorly understood, and systematic analyses based on comprehensive evaluation are lacking.

In this study, 3-year-old single-stem bud sticks of ‘Reken 628’ grafted onto GT1 seedling rootstocks were used to

evaluate the effects of irrigation frequency and leaf whorl position. Morphological and physiological traits of stems, leaves, and buds were measured to clarify response patterns and identify the optimal treatment combination. The results provide a scientific basis for efficient propagation of improved rubber tree varieties.

II. MATERIAL AND METHODS

Experimental site The experiment was conducted at the Rubber Tree Seedling Budding Demonstration Base of the Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, located in Danzhou City, Hainan Province (109°29'E, 19°30'N, altitude 116.9 m). The area has a tropical monsoon climate. During the experimental period, the annual average temperature ranged from 21 to 30 °C, the annual average precipitation was 1652.3 mm, and the annual average sunshine duration was 1734 h, which is suitable for rubber tree growth [8].

Experimental Materials Three-year-old single-stem bud sticks of ‘Reken 628’ on GT1 seedling rootstocks were

used. GT1 rootstocks were sown in October 2021, and budding was performed in October 2022. After sequential cutting back and shaping, uniform plants with four leaf whorls were obtained in September 2024. Uniform water and fertilizer management and plant protection measures were applied throughout the experimental period.

Experimental Design A two-factor split-plot design was used, with irrigation frequency as the main plot and leaf whorl position as the subplot. Six treatments were arranged with three replications and four plants per replicate.

Irrigation frequency: A1 = once daily at 08:00; A2 = twice daily at 08:00 and 18:00; 2 L per plant per application.

Leaf whorl position: B1 = 2nd reverse leaf whorl (from the top); B2 = 3rd reverse leaf whorl (from the top); B3 = 4th reverse leaf whorl (from the top).

Soil water content: 60%–70% in A1 and 75%–85% in A2. No leaves were removed during the experiment, and the photoperiod was 12 h.

Table 1 Experiment design

Treatment	Irrigation frequency	Leaf whorl position	Soil water content	Irrigation volume
A1B1	once-daily	2 nd reverse leaf whorl (from the top)	60%–70%	2 L per time
A1B2	once-daily	3 rd reverse leaf whorl (from the top)	60%–70%	2 L per time
A1B3	once-daily	4 th reverse leaf whorl (from the top)	60%–70%	2 L per time
A2B1	twice-daily	2 nd reverse leaf whorl (from the top)	75%–85%	2 L per time
A2B2	twice-daily	3 rd reverse leaf whorl (from the top)	75%–85%	2 L per time
A2B3	twice-daily	4 th reverse leaf whorl (from the top)	75%–85%	2 L per time

Experimental Methods After the experiment, indicators of bud sticks in each treatment group were measured as follows:

Morphological indicators: Leaf length and leaf width were measured with a ruler (precision 0.1 cm); plant height was measured with a tape measure (precision 0.1 cm); leaf number was counted directly. Length, width and thickness of scale bud scars, length and width of scale bud eyes, length, width and thickness of petiole bud scars, and length and width of petiole bud eyes were measured with a vernier caliper (precision 0.01 mm). Number of scale buds and number of petiole buds were counted directly. Stem diameter was measured with a vernier caliper at 30 cm above the base of bud sticks (precision 0.01 mm).

Physiological indicators: Leaf water content, stem water content, scale bud water content and petiole bud water content were determined by the drying-weighing method. Fresh samples were weighed (fresh weight W1), dried to

constant weight at 60 °C and weighed (dry weight W2). Water content = $(W1 - W2) / W1 \times 100\%$.

Bud eye damage rate: Total number of bud eyes in each treatment was counted. Bud eyes with depression, discoloration or mechanical damage were recorded as damaged bud eyes. Damage rate = $(\text{number of damaged bud eyes} / \text{total number of bud eyes}) \times 100\%$.

Data processing and analysis ANOVA was performed using DPS 20.05; Spearman correlation analysis was conducted using Origin 2024; comprehensive evaluation was carried out using the entropy-weighted TOPSIS method and PCA.

III. RESULT AND ANALYSIS

Main and Interactive Effects

As shown in Figure 1a, the leaf water content of the A1B3 (mean 63.33%), A1B2 (mean 63%), A1B1 (mean 62.67%),

and A2B1 (mean 62%) were significantly higher than that of the A2B3 (mean 55.67%) by 13.76% (difference 7.66), 13.17% (difference 7.33), 12.57% (difference 7.00), and 11.37% (difference 6.33), respectively. No significant differences were observed among the other treatments.

As shown in Figure 1b, the scale bud eye length of the A1B1 (mean 2.97 mm) was significantly longer than that of A1B3 (mean 2.1967 mm) by 35.20% (difference 0.7733) and significantly longer than that of the A2B3 treatment (mean 2.1667 mm) by 37.07% (difference 0.8033). Meanwhile, the scale bud eye lengths of the A2B1 treatment (mean 2.84 mm) and A2B2 treatment (mean

2.85 mm) were significantly longer than that of the A2B3 treatment by 31.07% (difference 0.6733) and 31.69% (difference 0.6833), respectively. No significant differences were detected among the other treatments.

Irrigation frequency significantly affected leaf water content and scale bud eye length, and a significant interaction between irrigation frequency and leaf whorl position was detected. Under twice daily irrigation, the 2nd and 3rd reverse leaf whorls exhibited significantly higher leaf water content and longer bud eyes than the 4th whorl. At the same leaf position, A2 was significantly superior to A1.

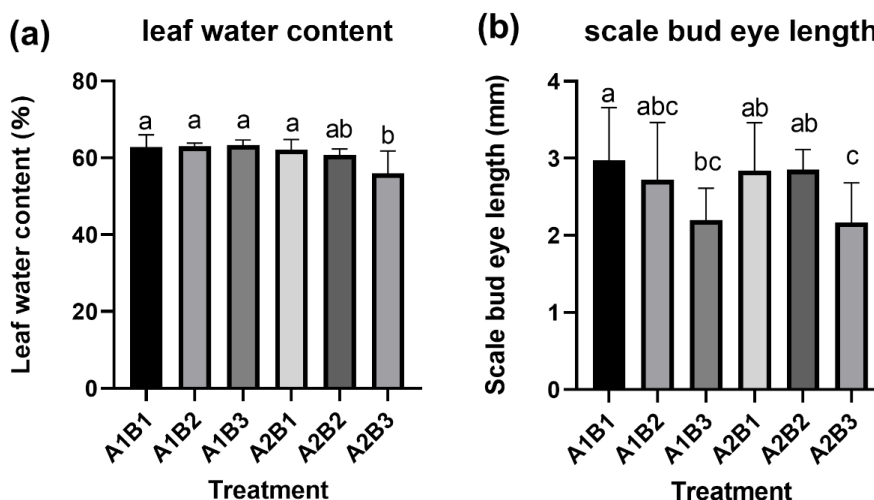


Fig.1 Effects of irrigation frequency and leaf whorl position on leaf water content and scale bud eye length in 'Reken 628' bud sticks

Note: Different lowercase letters indicate significant differences among treatments at $P < 0.05$; error bars represent standard deviation, $n = 3$. A1: once daily irrigation; A2: twice daily irrigation; B1: 2nd reverse leaf whorl; B2: 3rd reverse leaf whorl; B3: 4th reverse leaf whorl.

Trait Correlation Analysis

Figure 2 shows the correlations among various indices of stem, leaf, and axillary bud tissues of *Hevea brasiliensis* 'Reken 628' bud sticks under different watering frequencies (once daily, twice daily) and leaf whorl positions (2nd, 3rd, 4th canopies from the top).

Under the A1B1 treatment (Figure 2A), plant height was significantly positively correlated with leaf water content and highly significantly positively correlated with petiole bud scar width; it also showed highly significant positive correlations with leaf length, scale bud eye length, scale bud eye width, petiole bud eye length, and petiole bud eye width, respectively. Stem diameter was highly significantly negatively correlated with bud eye damage rate, and significantly positively correlated with leaf number, scale bud scar length, and petiole bud scar length,

respectively. Leaf number was significantly positively correlated with petiole bud scar length and significantly negatively correlated with bud eye damage rate. Leaf length was significantly positively correlated with petiole bud scar width, and highly significantly positively correlated with leaf width, scale bud eye length, scale bud eye width, petiole bud eye length, and petiole bud eye width, respectively. Leaf width was significantly positively correlated with scale bud eye length, petiole bud eye length, and petiole bud eye width, respectively. Leaf water content was significantly positively correlated with scale bud eye length, scale bud eye width, and petiole bud eye width, respectively. Scale bud number was significantly positively correlated with scale bud scar length, scale bud eye length, scale bud eye width, petiole bud eye width, and petiole bud water content, respectively.

Scale bud scar length was significantly positively correlated with petiole bud water content and significantly negatively correlated with bud eye damage rate. Scale bud eye length was significantly positively correlated with petiole bud scar width, and highly significantly positively correlated with scale bud eye width, petiole bud eye length, and petiole bud eye width, respectively. Scale bud eye width was significantly positively correlated with petiole bud scar width, and highly significantly positively correlated with petiole bud eye length and petiole bud eye width, respectively. Scale bud water content was significantly positively correlated with petiole bud number. Petiole bud scar width was significantly positively correlated with petiole bud eye length and petiole bud eye width, respectively. Petiole bud eye length was highly significantly positively correlated with petiole bud eye width.

Under the A1B2 treatment (Figure 2B), plant height was significantly positively correlated with stem diameter, leaf water content, and scale bud number, respectively. Stem diameter was highly significantly positively correlated with leaf number and scale bud scar length, and also highly significantly positively correlated with petiole bud number. Leaf number was significantly positively correlated with petiole bud number. Leaf length was significantly positively correlated with leaf width. Leaf width was significantly positively correlated with leaf water content, scale bud number, and scale bud scar thickness, respectively, and highly significantly positively correlated with petiole bud scar width. Leaf water content was significantly positively correlated with scale bud number and petiole bud eye width, respectively. Scale bud number was significantly positively correlated with scale bud eye length and petiole bud scar width, respectively. Scale bud scar length was significantly positively correlated with scale bud water content and petiole bud water content, respectively, and highly significantly negatively correlated with bud eye damage rate. Scale bud scar width was extremely significantly positively correlated with scale bud eye width, highly significantly positively correlated with scale bud scar thickness, and significantly positively correlated with scale bud eye length, petiole bud scar width, petiole bud eye length, and petiole bud eye width, respectively. Scale bud scar thickness was highly significantly positively correlated with petiole bud scar width, and significantly positively correlated with scale bud eye width and petiole bud eye width, respectively. Scale bud eye length was extremely significantly positively correlated with petiole bud eye length, highly significantly positively correlated with scale bud eye width, and significantly positively correlated with petiole bud eye width. Scale bud eye width

was highly significantly positively correlated with petiole bud scar width, and significantly positively correlated with petiole bud eye length. Scale bud water content was highly significantly positively correlated with petiole bud water content, and significantly negatively correlated with bud eye damage rate. Petiole bud scar length was significantly negatively correlated with bud eye damage rate. Petiole bud scar thickness was significantly positively correlated with petiole bud eye width. Petiole bud eye length was significantly positively correlated with petiole bud eye width. Petiole bud water content was significantly negatively correlated with bud eye damage rate.

Under the A1B3 treatment (Figure 2C), plant height was significantly negatively correlated with scale bud scar length and petiole bud scar length, respectively. Stem diameter was significantly positively correlated with scale bud water content. Leaf number was highly significantly positively correlated with leaf length and leaf width, respectively. Leaf length was extremely significantly positively correlated with leaf width. Leaf water content was extremely significantly positively correlated with petiole bud scar width, and significantly positively correlated with scale bud eye width and petiole bud eye width, respectively. Scale bud number was significantly negatively correlated with petiole bud scar length and petiole bud water content, respectively. Scale bud scar length was significantly positively correlated with scale bud scar width and petiole bud eye length, respectively. Scale bud scar width was significantly negatively correlated with petiole bud number, highly significantly positively correlated with petiole bud eye length, and significantly positively correlated with petiole bud scar width and petiole bud eye width, respectively. Scale bud scar thickness was highly significantly negatively correlated with scale bud water content. Scale bud eye length was significantly negatively correlated with petiole bud number. Scale bud eye width was significantly positively correlated with petiole bud eye width. Petiole bud number was significantly negatively correlated with petiole bud eye length, highly significantly negatively correlated with bud eye damage rate, and highly significantly positively correlated with petiole bud scar width and petiole bud eye width.

Under the A2B1 treatment (Figure 2D), plant height was significantly positively correlated with leaf number, scale bud scar thickness, petiole bud number, petiole bud scar length, petiole bud scar thickness and petiole bud eye length, respectively, and highly significantly positively correlated with stem diameter, scale bud scar thickness, scale bud eye length, petiole bud scar width and petiole bud eye width, respectively; stem diameter was significantly positively correlated with petiole bud scar

width, petiole bud eye length and petiole bud eye width, respectively, highly significantly positively correlated with leaf number, leaf width, scale bud scar width, petiole bud number and petiole bud scar length, respectively, and extremely significantly positively correlated with scale bud scar thickness; stem water content was significantly positively correlated with leaf water content; leaf number was significantly positively correlated with scale bud scar thickness, petiole bud scar length, petiole bud scar width, petiole bud eye length and petiole bud eye width, respectively, highly significantly positively correlated with petiole bud number and petiole bud scar thickness, and extremely significantly positively correlated with leaf width; leaf length was significantly positively correlated with scale bud scar length, and highly significantly positively correlated with leaf width and scale bud eye width, respectively; leaf width was significantly positively correlated with petiole bud number; leaf water content was significantly negatively correlated with scale bud number; scale bud scar length was extremely significantly positively correlated with scale bud eye width and highly significantly positively correlated with petiole bud eye width; scale bud scar width was significantly positively correlated with scale bud eye length and petiole bud scar thickness, respectively, and highly significantly positively correlated with scale bud scar thickness, petiole bud number, petiole bud scar width, petiole bud eye length and petiole bud eye width, respectively; scale bud scar thickness was significantly positively correlated with scale bud eye length, highly significantly positively correlated with petiole bud scar length, petiole bud scar thickness and petiole bud eye length, respectively, and extremely significantly positively correlated with petiole bud number; scale bud eye length was significantly positively correlated with scale bud eye width, petiole bud number, petiole bud eye length and petiole bud eye width; scale bud eye width was significantly positively correlated with petiole bud eye width; scale bud water content was significantly positively correlated with petiole bud water content; petiole bud number was significantly positively correlated with petiole bud scar width and petiole bud eye width, highly significantly positively correlated with petiole bud scar thickness, and extremely significantly positively correlated with petiole bud scar length and petiole bud eye length, respectively; petiole bud scar length was significantly positively correlated with petiole bud scar width and petiole bud eye width, highly significantly positively correlated with petiole bud scar thickness, and extremely significantly positively correlated with petiole bud eye length; petiole bud scar width was significantly positively correlated with petiole bud eye length, and highly significantly positively correlated with petiole bud scar

thickness and petiole bud eye width, respectively; petiole bud scar thickness was highly significantly positively correlated with petiole bud eye length; petiole bud eye length was highly significantly positively correlated with petiole bud eye width.

Under the A2B2 treatment (Figure 2E), plant height was significantly positively correlated with leaf length, leaf width, petiole bud scar length and petiole bud scar width, highly significantly positively correlated with scale bud eye length, scale bud eye width and petiole bud eye width, respectively, and extremely significantly positively correlated with scale bud scar length and petiole bud eye length; stem diameter was highly significantly positively correlated with leaf number and petiole bud scar thickness, and extremely significantly positively correlated with scale bud scar width and scale bud scar thickness; stem water content was significantly positively correlated with leaf length, leaf width, scale bud scar length and petiole bud scar width, respectively; leaf number was significantly positively correlated with petiole bud eye length, highly significantly positively correlated with scale bud scar thickness and petiole bud scar thickness, and extremely significantly positively correlated with scale bud scar width; leaf length was highly significantly positively correlated with leaf width and petiole bud scar width, and extremely significantly positively correlated with petiole bud scar length; leaf width was significantly positively correlated with petiole bud scar width and petiole bud scar thickness, and highly significantly positively correlated with petiole bud scar length; scale bud number was highly significantly positively correlated with scale bud water content; scale bud scar length was highly significantly positively correlated with scale bud eye width and petiole bud eye width, and extremely significantly positively correlated with scale bud eye length and petiole bud eye length; scale bud scar width was highly significantly positively correlated with petiole bud scar thickness, and extremely significantly positively correlated with scale bud scar thickness; scale bud scar thickness was extremely significantly positively correlated with petiole bud scar thickness; scale bud eye length was significantly positively correlated with scale bud eye width and petiole bud eye width, and highly significantly positively correlated with petiole bud eye length; scale bud eye width was extremely significantly positively correlated with petiole bud eye length and petiole bud eye width; petiole bud scar length was significantly positively correlated with petiole bud scar thickness, and highly significantly positively correlated with petiole bud scar width; petiole bud eye length was extremely significantly positively correlated with petiole bud eye width.

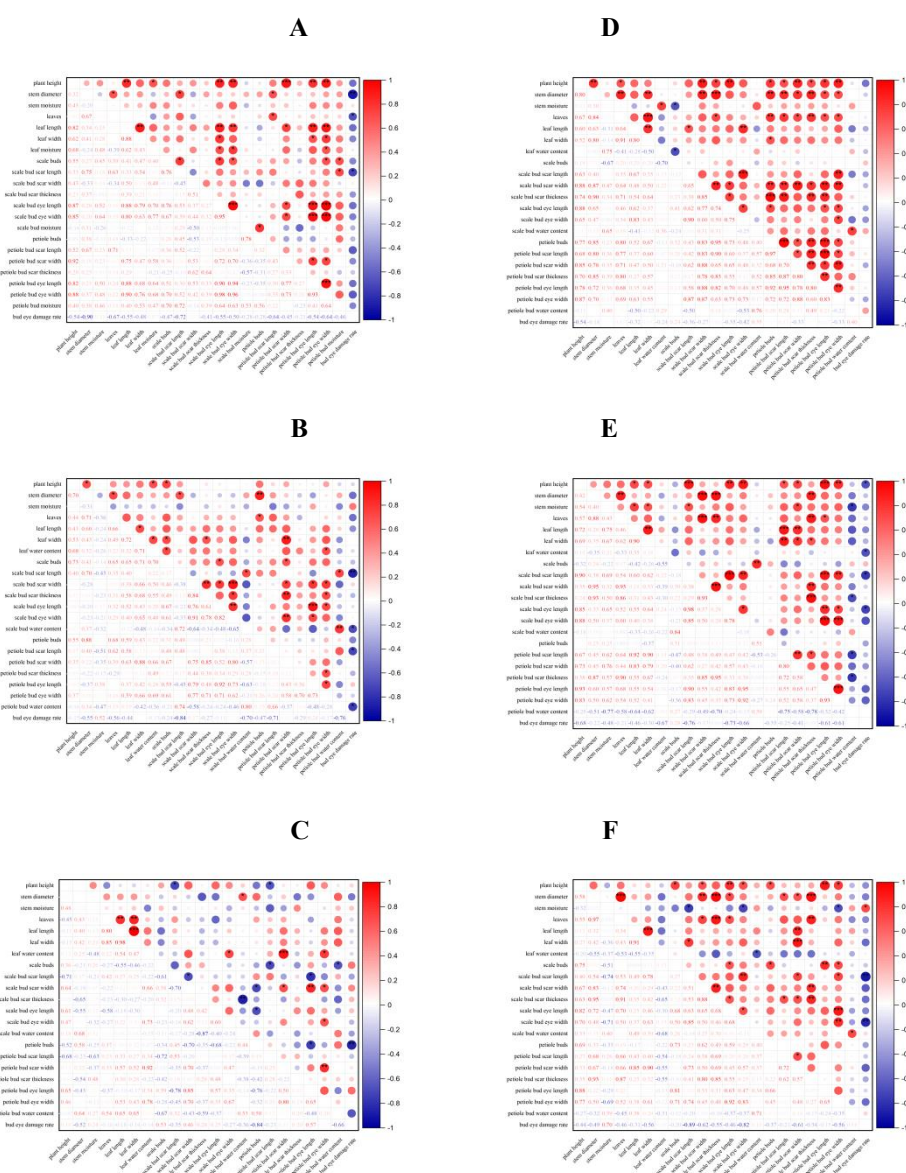


Fig.2 Correlation heatmap of measured traits across treatments

Note: A–C represent the correlation analysis among morphological indices of stems and leaves under A1B1, A1B2, and A1B3, respectively. D–F represent the correlation analysis among morphological indices of stems and leaves under A2B1, A2B2, and A2B3, respectively. The size and color depth of the circles indicate the magnitude of the correlation coefficient and the significance level, respectively. Blue represents negative correlation and red represents positive correlation. The upper triangle shows significance markers, where *, **, and *** indicate statistically significant correlation at $p < 0.05$, highly significant correlation at $p < 0.01$, and extremely significant correlation at $p < 0.001$, respectively. The lower triangle shows the values of correlation coefficients, where $|r| \geq 0.7$ indicates strong correlation, $0.3 \leq |r| < 0.7$ indicates moderate correlation, and $|r| < 0.3$ indicates weak correlation.

Under the A2B3 treatment (Figure 2F), plant height was significantly positively correlated with scale bud number, scale bud scar width, scale bud eye width, petiole bud number and petiole bud eye width, respectively, and highly significantly positively correlated with scale bud eye length and petiole bud eye length; stem diameter was significantly positively correlated with scale bud eye

length, petiole bud scar length and petiole bud scar width, highly significantly positively correlated with scale bud scar width, and extremely significantly positively correlated with leaf number, scale bud scar thickness and petiole bud scar thickness, respectively; stem water content was significantly negatively correlated with scale bud scar length, scale bud eye width and petiole bud eye width,

respectively, and significantly positively correlated with bud eye damage rate; leaf number was significantly positively correlated with scale bud scar width and scale bud eye length, highly significantly positively correlated with petiole bud scar thickness, and extremely significantly positively correlated with scale bud scar thickness; leaf length was highly significantly positively correlated with petiole bud scar width, and extremely significantly positively correlated with leaf width; leaf width was significantly positively correlated with scale bud scar length, and extremely significantly positively correlated with petiole bud scar width; leaf water content was significantly negatively correlated with scale bud water content; scale bud number was significantly positively correlated with scale bud eye length, petiole bud number and petiole bud eye width, and highly significantly positively correlated with petiole bud eye length; scale bud scar length was significantly positively correlated with petiole bud scar width and petiole bud eye width, highly significantly negatively correlated with bud eye damage rate, and highly significantly positively correlated with scale bud eye width; scale bud scar width was highly significantly positively correlated with scale bud scar thickness and petiole bud scar thickness; scale bud scar thickness was significantly positively correlated with scale bud eye length, petiole bud scar length and petiole bud scar width, respectively, and highly significantly positively correlated with petiole bud scar thickness; scale bud eye length was significantly positively correlated with scale bud eye width, and extremely significantly positively correlated with petiole bud eye width; scale bud eye width was highly significantly positively correlated with petiole bud eye width, and highly significantly negatively correlated with bud eye damage rate; scale bud water content was significantly positively correlated with petiole bud water content; petiole bud scar length was significantly positively correlated with petiole bud scar width.

Overall, watering frequency and leaf whorl position significantly affected the correlation pattern among indicators: most growth indices showed a synergistic enhancement relationship under A2B2 treatment; while under A1B4, plant height was negatively correlated with bud scar length, indicating that excessive leaf removal may inhibit longitudinal growth.

TOPSIS Comprehensive Evaluation

Raw measurement data were transformed by logarithm. Plant height, stem diameter, bud eye damage rate, scale bud scar thickness and petiole bud scar thickness were taken as negative indicators, while the weights of other indicators (leaf number, leaf water content, stem water content, leaf length, leaf width, scale bud scar length, scale

bud scar width, scale bud eye length, scale bud eye width, scale bud number, scale bud water content, petiole bud scar length, petiole bud scar width, petiole bud eye length, petiole bud eye width, petiole bud number, petiole bud water content, etc.) were determined by the entropy weight method. The TOPSIS method was used for analysis, and the results are shown in Table 2.

When considering watering frequency, the axillary bud quality under twice-daily watering was superior to that under once-daily watering. When ignoring watering frequency and only considering leaf whorl position, the axillary bud quality at the 2nd leaf whorl was the best, followed by the 3rd leaf whorl, and the 4th leaf whorl was the lowest. Considering both watering frequency and leaf whorl position comprehensively, the axillary bud quality was optimal under A2B1, followed by A2B2, and A2B3. Under once-daily watering, the axillary bud quality at the 3rd leaf whorl was the best, followed by the 4th leaf whorl, and the 2nd leaf whorl was the lowest. The ranking of axillary bud quality was as follows: A2B1 (best), A2B2(second best), and the 3rd to 6th places were A2B3, A1B2, A1B3, and A1B1, respectively. From the perspective of application prospects: in the practice of bud selection for budding of *Hevea brasiliensis* ‘Reken 628’, the axillary bud quality at all leaf whorl positions under twice-daily watering was better than that under once-daily watering. However, on the premise of preferentially selecting axillary buds from the 2nd leaf whorl and then the 3rd leaf whorl, once-daily watering can still serve as a supplementary option to twice-daily watering, providing a feasible water management and leaf whorl position matching scheme for budding production.

Table 2 TOPSIS comprehensive analysis of morphological indices based on leaf, stem and axillary bud tissues

Treatment	Comprehensive index (CI)	Rank
A2B1	0.7785	1
A2B2	0.3502	2
A2B3	0.3030	3
A1B2	0.2250	4
A1B3	0.2035	5
A1B1	0.1998	6

Note: CI, approximation to the Optimal Vectors.

Principal Component Analysis

Principal component analysis (PCA) was performed on 22 morphological indices related to axillary bud quality of *Hevea brasiliensis* ‘Reken 628’ bud sticks, including traits

related to leaves, stems and axillary buds (Figure 3). The eigenvalues of the first two principal components were 9.630 and 6.508, with variance contribution rates of 43.8% and 29.6%, respectively, and a cumulative contribution rate of 73.4%. This indicates that PC1 and PC2 can effectively summarize most of the information in the original data, and the index system achieves a satisfactory dimension reduction effect, allowing comprehensive evaluation of axillary bud quality under different watering frequency–leaf whorl position treatments.

Variance Explanation and Principal Component Extraction The eigenvalue of PC1 was 9.62965, which independently explained 43.8% of the variation in the original data, representing the core principal component reflecting axillary bud quality. The eigenvalue of PC2 was 6.50816, explaining 29.66% of the original data variation as the secondary principal component. Their cumulative contribution rate exceeded 70%, meeting the validity requirement for principal component analysis, so they can replace the original 22 indices for comprehensive evaluation.

Index Loading Distribution and Implications of Principal Components The main loading indices of PC1: indices with high positive loadings on PC1 included leaf width (LW, 0.29181), leaf number (L, 0.29929), scale bud eye length (SBEL, 0.29491), petiole bud scar length (LBSL, 0.28642), and petiole bud eye length (LBEL, 0.29163), all with loading values above 0.28. These indices showed strong positive correlations with PC1 and were core positive indicators for axillary bud quality and vegetative growth of bud sticks, reflecting plant vegetative organ vigor and axillary bud developmental potential. Indices with high negative loadings included stem diameter (SD, -0.2744) and scale bud scar thickness (SBST, -0.29823), which were predefined negative indicators (lower values expected) and negatively correlated with PC1, consistent with biological logic. Therefore, PC1 can be defined as the 'principal component of bud stick growth and axillary bud morphogenesis'. A higher PC1 score indicates better vegetative growth of bud sticks and superior morphological indices of axillary buds.

The main loading indices of PC2: indices with high positive loadings on PC2 included scale bud scar width (SBSW, 0.35976), scale bud eye width (SBEW, 0.37605), petiole bud number (LB, 0.29923), and leaf length (LL, 0.314), all with loading values above 0.29, mainly reflecting the spatial characteristics of bud eyes and axillary bud quantity. Indices with high negative loadings

included scale bud scar length (SBSL, -0.32894), leaf water content (LM, -0.30789), and petiole bud scar thickness (LBST, -0.245), which showed inhibitory effects on PC2. Therefore, PC2 can be defined as the 'principal component of axillary bud morphological details and water physiology'. A higher PC2 score indicates superior morphological details of axillary buds, such as bud eye and bud scar width.

Sample Score Distribution and Grouping Characteristics The comprehensive performance of the six different watering frequency–foliage whorl position treatments showed clear separation in the PC1–PC2 plane (Figure 3):

A2B1 (twice daily watering + 2nd leaf whorl): located in the first quadrant (positive PC1, positive PC2), with the highest PC1 score (1.256), indicating outstanding performance in "favorable indices" such as leaf number, leaf width and bud eye length. Meanwhile, the positive PC2 score (0.2744) reflected superior traits such as bud eye width. This treatment showed the best overall status in vegetative growth, axillary bud morphogenesis and morphological details, representing the optimal comprehensive performance.

A2B2 (twice daily watering + 3rd leaf whorl): also located in the first quadrant with positive PC1 (0.523) and PC2 (1.082) scores, but lower PC1 than A2B1, indicating slightly weaker vegetative growth indices, while the higher PC2 may be related to larger bud eye width.

A2B3 (twice daily watering + 4th leaf whorl): located in the second quadrant (negative PC1, positive PC2). The negative PC1 indicated poorer vegetative growth indices, while the positive PC2 suggested retained advantages in traits such as bud eye width.

A1B1, A1B2, A1B3 (once daily watering treatments): distributed in the third and fourth quadrants with mostly negative PC1 and PC2 scores, showing overall poor morphological performance. In particular, A1B3 (PC1 = -1.114, PC2 = -0.995), located in the lower-left corner, had the lowest comprehensive scores across all indices.

Overall, the PC1 scores of treatments with twice daily watering (A2 series) were superior to those with once daily watering (A1 series). For leaf whorl positions, the order was 2nd whorl (B1) > 3rd whorl (B2) > 4th whorl (B3), consistent with the practical cultivation rule that axillary buds in the middle-upper leaf whorls of bud sticks exhibit better quality.

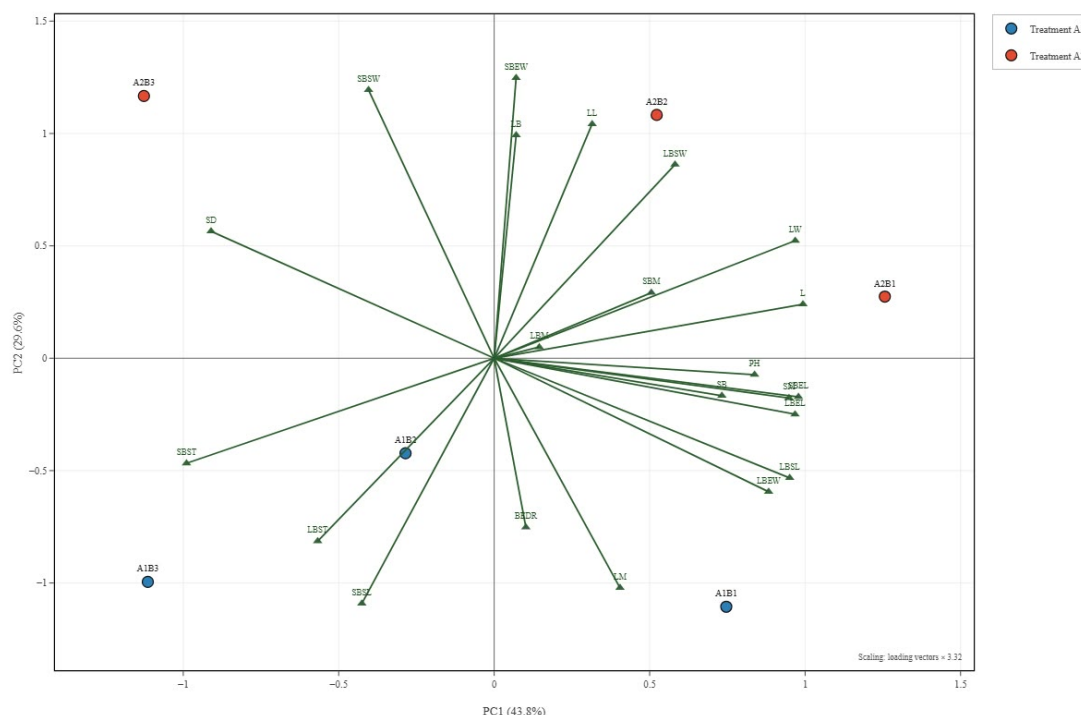


Fig.3. PCA biplot of treatments and traits. Scores (samples) are colored by treatment (A1: blue, A2: orange).

Note: Vectors (green arrows) represent loading values of 22 variables. The length and direction indicate contribution to PC1 and PC2. Variance explained: PC1 = 43.8% (eigenvalue 9.63), PC2 = 29.6% (eigenvalue 6.51), cumulative = 73.4%. Loadings scaling factor: 3.32 (relative arrow length).

IV. DISCUSSION AND CONCLUSION

DISCUSSION

Water supply status directly affects cell turgor, photosynthesis, and substance synthesis and transport in rubber tree bud sticks, thereby regulating stem and leaf growth as well as axillary bud development. In this experiment, the leaf water content of the 2nd and 3rd whorls under twice-daily watering was significantly higher than that of the 4th leaf whorl under the same watering frequency, indicating that appropriate water supply is more conducive to maintaining a relatively high water status in the middle-upper whorls, whereas excessive watering or relatively weak water metabolism capacity in lower canopies may occur, which is related to the physiological activity differences among different foliage canopies of rubber tree bud sticks. Under the tropical climate conditions in Danzhou, twice-daily watering can maintain a high and stable substrate water status, which is beneficial to sustaining cell turgor and photosynthate transport in bud sticks; in contrast, once-daily watering may cause water stress at noon and inhibit axillary bud differentiation. As an important morphological index of axillary bud development, scale bud eye length showed no significant difference between the 3rd leaf whorl under once-daily watering and the 2nd and 3rd whorls under twice-daily

watering, suggesting that the regulatory effect of watering frequency on axillary bud morphogenesis is leaf-whorl-specific, and the middle-upper whorls present more stable responses to water changes, providing a specific positional reference for precise water regulation in rubber tree bud stick cultivation.

The significant correlations among various indices of bud sticks revealed the internal relationship between stem-leaf growth and axillary bud development. Leaf width was extremely significantly positively correlated with leaf water content, indicating that larger leaf morphology confers stronger water retention capacity and thus sufficient water supply for axillary bud development. Meanwhile, leaf water content was extremely significantly negatively correlated with scale bud scar thickness, and it is speculated that higher leaf water content may inhibit lignification of bud scar tissues, favoring axillary bud germination and growth. This conclusion is consistent with the response pattern of osmotic adjustment substances in different organs of rubber seedlings to water reported by Cheng *et al.* [9], confirming the consistent regulatory effect of water metabolism on organ morphogenesis in rubber tree seedlings. Stem water content was positively correlated with scale bud scar length and negatively correlated with scale bud scar width, reflecting the

differential regulation of bud scar morphology by stem water metabolism; sufficient stem water can promote longitudinal growth of bud scars, providing more space for axillary bud development[10-11]. In addition, scale bud eye length was significantly positively correlated with both petiole bud scar length and petiole bud eye length, indicating coordinated morphological development of axillary buds, with mutual promotion between bud eye and bud scar growth. This is consistent with the findings of Jiang *et al.* [4] on the correlation between axillary bud quality and plant morphological indices in 'Reken 628', highlighting the morphological coordination characteristic of axillary bud development in this variety.

The comprehensive evaluation results using the TOPSIS method showed that A2B1 treatment was the optimal treatment for axillary bud quality. This is because twice-daily watering maintains stable water metabolism in bud sticks, and their synergistic effect promotes the formation of high-quality axillary buds. As a functional whorl in the middle-upper part of bud sticks, the 2nd leaf whorl has high physiological activity and strong nutrient supply capacity, so its axillary bud quality is significantly superior to that of the 3rd and 4th whorls. This is consistent with the apical dominance and nutrient distribution rules of rubber tree bud sticks, and also provides experimental support for the findings of Cha *et al.* [5–6] regarding the effects of leaf whorl position on axillary bud quality in umbrella-shaped 'Reken 628' bud sticks. The overall axillary bud quality under once-daily watering was inferior to that under twice-daily watering, indicating that appropriately increasing watering frequency is conducive to improving axillary bud quality during the cultivation of 'Reken 628' bud sticks. However, the axillary bud quality of the 3rd leaf whorl under once-daily watering remained relatively favorable and can serve as a supplement to the optimal treatment, providing a flexible scheme for bud selection under different water management conditions.

The comprehensive scores were calculated by weighting the scores of the two principal components in PCA according to variance ($PC1 \times 44.8\% + PC2 \times 30.3\%$) and ranked as follows: A2B1 (0.654) > A2B2 (0.560) > A1B1 (0.000) > A2B3 (-0.154) > A1B2 (-0.256) > A1B3 (-0.803). This order partially differs from the TOPSIS ranking (A2B1 > A2B2 > A2B3 > A1B2 > A1B3 > A1B1), mainly reflected in the swapped positions of A1B1 and A2B3. The PCA weighted score places greater emphasis on the weight of PC1 (vegetative growth), whereas TOPSIS objectively assigns weights to 22 indices via the entropy weight method, which may assign higher weights to bud eye morphological indices, resulting in a higher ranking for A2B3 (with a higher PC2 score) than A1B1 (with a higher PC1 score but extremely low PC2

score). This difference precisely reflects the different focuses of the two methods: PCA is a linear dimensionality-reduction technique, while TOPSIS is a multi-objective decision-making method. The latter is more consistent with the comprehensive trade-off between 'favorable indices' and 'negative indices' in actual production, indicating that the TOPSIS results are reliable and can be used to guide water management and leaf whorl matching strategies in bud selection for 'Reken 628' rubber tree budding.

This study only set two watering frequencies and focused solely on the three-year-old single-stem bud sticks of 'Reken 628', without covering different tree ages, seasons or soil types. In the future, multi-level gradient experiments should be carried out, combined with data on budding survival rate and seedling growth, to further verify the optimal water management scheme.

V. CONCLUSION

In this study, three-year-old single-stem bud sticks of *Hevea brasiliensis* 'Reken 628' were used as experimental materials to systematically investigate the effects of two watering frequencies (once daily and twice daily) combined with different leaf whorl positions on the morphology, physiology and axillary bud quality of bud sticks. Through analysis of variance, correlation analysis and comprehensive evaluation using the TOPSIS method, and PCA analysis, the core conclusions are as follows:

Watering frequency and leaf whorl position have a significant interactive effect on the axillary bud quality of 'Reken 628' rubber tree bud sticks. The 2nd and 3rd leaf whorls show more stable responses to water, while the 4th leaf whorl exhibits the poorest performance. Stem-leaf growth and axillary bud development of bud sticks are highly correlated, and leaf water content and leaf width are key indices affecting axillary bud morphogenesis. The A2B1 treatment yields the optimal axillary bud quality. Although the A1B3 overall axillary bud quality is lower than that of twice-daily watering treatments, it can be used as a suboptimal alternative in production scenarios with limited water resources or insufficient irrigation facilities. Appropriately increasing watering frequency and selecting axillary buds from middle-upper leaf whorls can significantly improve the quality of budding seedling propagation.

ACKNOWLEDGEMENTS

This work was supported by Hainan Provincial Natural Science Foundation of China(326MS0289), the earmarked

fund for China Agriculture Research System (CARS-34-YZ4).

REFERENCES

- [1] Yang YW, Xiong YL, Gao XJ, Yin YQ, Zhang FL(2026).Regional trial analysis of eight excellent rubber tree varieties in xishuangbanna. Tropical Agricultural Science and Technology, 4: 1-6.
- [2] Wang XJ, Huang X, Zhang YY, Li WG, XF Zhang XF, XS Gao XS, Zhang HL(2025) Differences in understory light conditions among rubber plantations of different strains and their causes. Chinese Journal of Tropical Crops, 46(12): 100-111.
- [3] Yang HZ, Li WP, Chen XH, Wang J (2026) Influence of axillary bud types on the branching angle of new shoots and the growth quality of bud sticks in rubber tree 'Reken 628'. International Journal of Environment, Agriculture and Biotechnology, 11(1), 108–122.
- [4] Jiang XY, Gong XD, Ruan JL, Cha ZN, Chen XH, Jun W (2026) Effects of Cutting-Propagated Rootstocks on Axillary Bud Quality of *Hevea brasiliensis* 'Reken 628'. China Tropical Agriculture, 1: 70-79.
- [5] Cha ZN, Ruan JL, Jiang XY, Gong XD, Chen XH , Wang J(2025) Rubber tree 'Reken 628' single-stem bud stick leaf-clipped treatment improves seedling axillary bud quality, International Journal of Innovative Science and Research,78(1): 1–13.
- [6] Cha ZN, Ruan JL, Jiang XY, Gong XD, Chen XH, Wang J(2025) Effects of leaf reserved and clipped on axillary bud quality in umbrella-shaped *Hevea brasiliensis* 'Reken 628'bud-sticks. International Journal of Environment, Agriculture and Biotechnology 10(2):48–59.
- [7] Ruan JL, Cha ZN , Gong XD, Jiang XY, Chen XH, Wang J(2024) Light intensity affects axillary bud quality of rubber mini-seedling budding CATAS73397, International Journal of Environment, Agriculture and Biotechnology, 9(6): 166–179.
- [8] Chen XM, Li WG, Chen HL, Liu SJ, Zhang JH, Wu QL (2019) Establishment and application of climate suitability evaluation index for rubber tapping in Hainan Island: Taking Danzhou City as an example. Jiangsu Agricultural Sciences, 47(15): 278–281.
- [9] Cheng YW, An F, Shi JR, Xie GS, Cai J (2022) Composition of osmotica in different organs of rubber seedlings and their contributions to osmotic potential. Journal of Tropical Biology, 13(1): 7–12.
- [10] Adahé LNA., Kouadio VPG., Abrou EJN., Yao ON., Kossonou FSA, Tondoh JE, Yao CYA (2024) Plant species diversity in cocoa and rubber tree landscapes in Côte d'Ivoire. International Journal of Biological and Chemical Sciences, 17(6):2377-2395.
- [11] Gao XS, Hu XX, Li T, Li WG, Huang X (2021). Research on characteristics of leaf anatomical structure and photosynthesis of 5 widely-used *Hevea brasiliensis* clones seedlings. Journal of Southwest Forestry University (Natural Science), 41(3): 31–36.